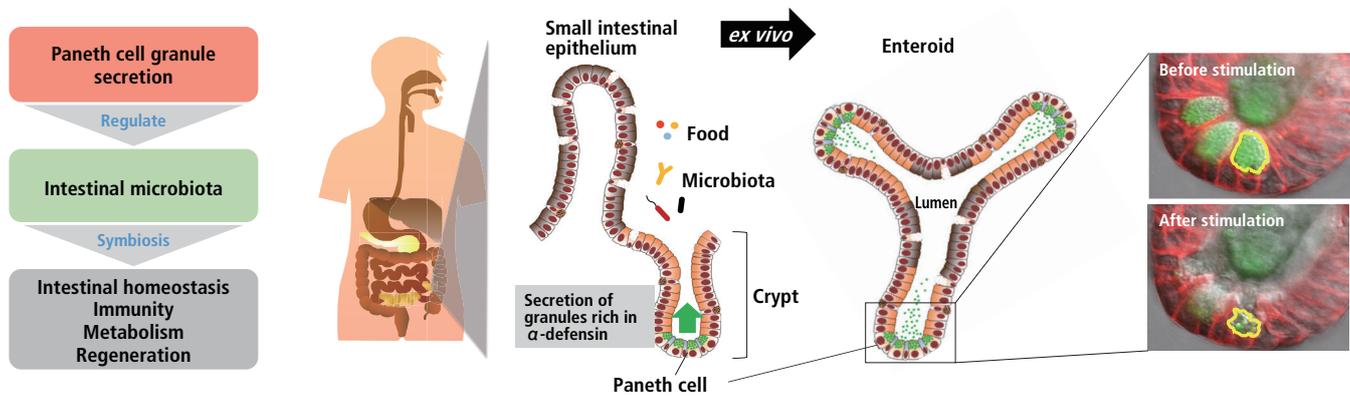


# Live Imaging of Paneth Cell Secretory Responses in Innate Immunity by Using Three-Dimensional Culture of Small Intestinal Epithelial Cells

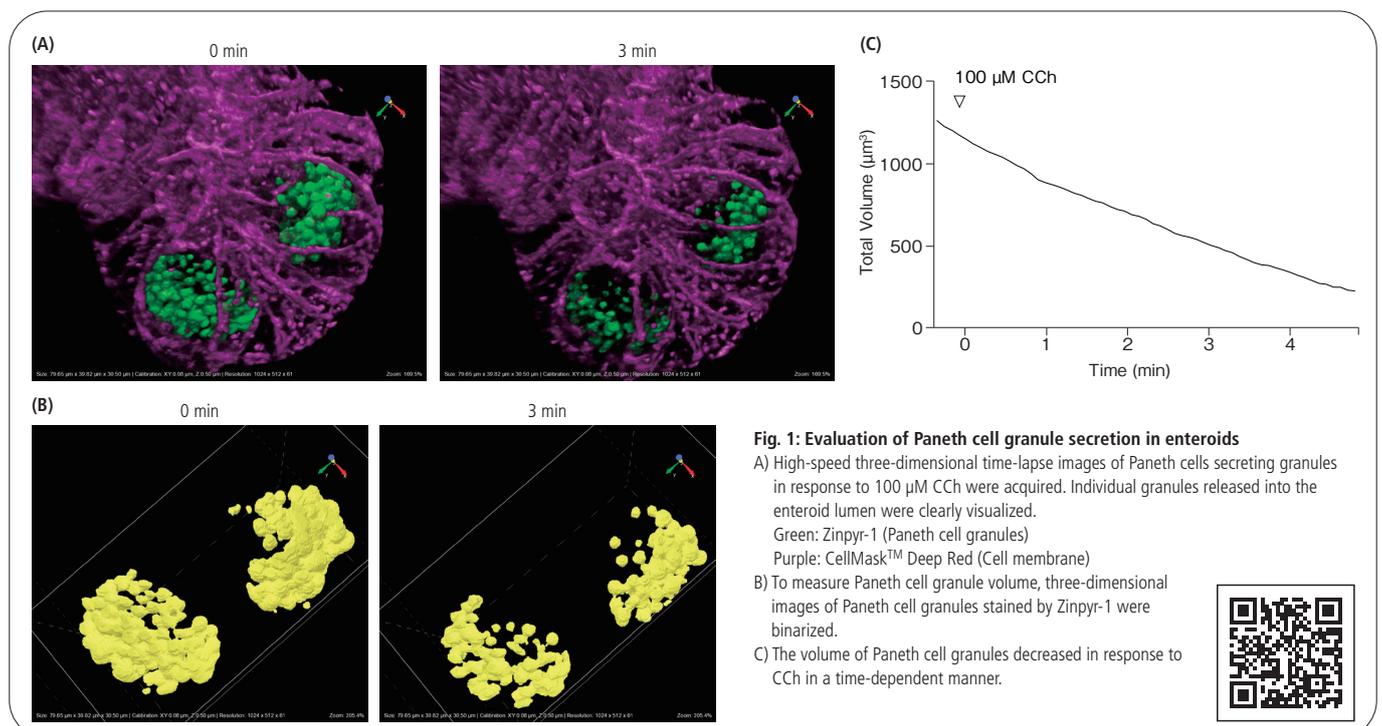
Paneth cells, a lineage of small intestinal epithelial cells, secrete granules rich in antimicrobial peptides,  $\alpha$ -defensins, in response to cholinergic agents and bacteria, and regulate the intestinal microbiota by killing enteric pathogens, while less killing commensal bacteria. In this Application Note, we introduce examples that clarify the mechanisms of  $\alpha$ -defensin secretion by visualization and quantification of Paneth cell granule secretory responses *ex vivo* using enteroid, a three-dimensional culture system of small intestinal epithelial cells.



## Three-dimensional time-lapse analysis of Paneth cell granule secretion

Carbachol (CCh), a cholinergic agent, was added to enteroids, and Paneth cell immune responses were observed.

Paneth cell granule secretion toward the lumen of the enteroids was evaluated by visualization and quantification using high-speed 3D time-lapse imaging with a high-speed resonant scanner and a piezo Z stage (Fig. 1).



**Fig. 1: Evaluation of Paneth cell granule secretion in enteroids**

A) High-speed three-dimensional time-lapse images of Paneth cells secreting granules in response to 100  $\mu$ M CCh were acquired. Individual granules released into the enteroid lumen were clearly visualized.

Green: Zinpyr-1 (Paneth cell granules)  
Purple: CellMask™ Deep Red (Cell membrane)

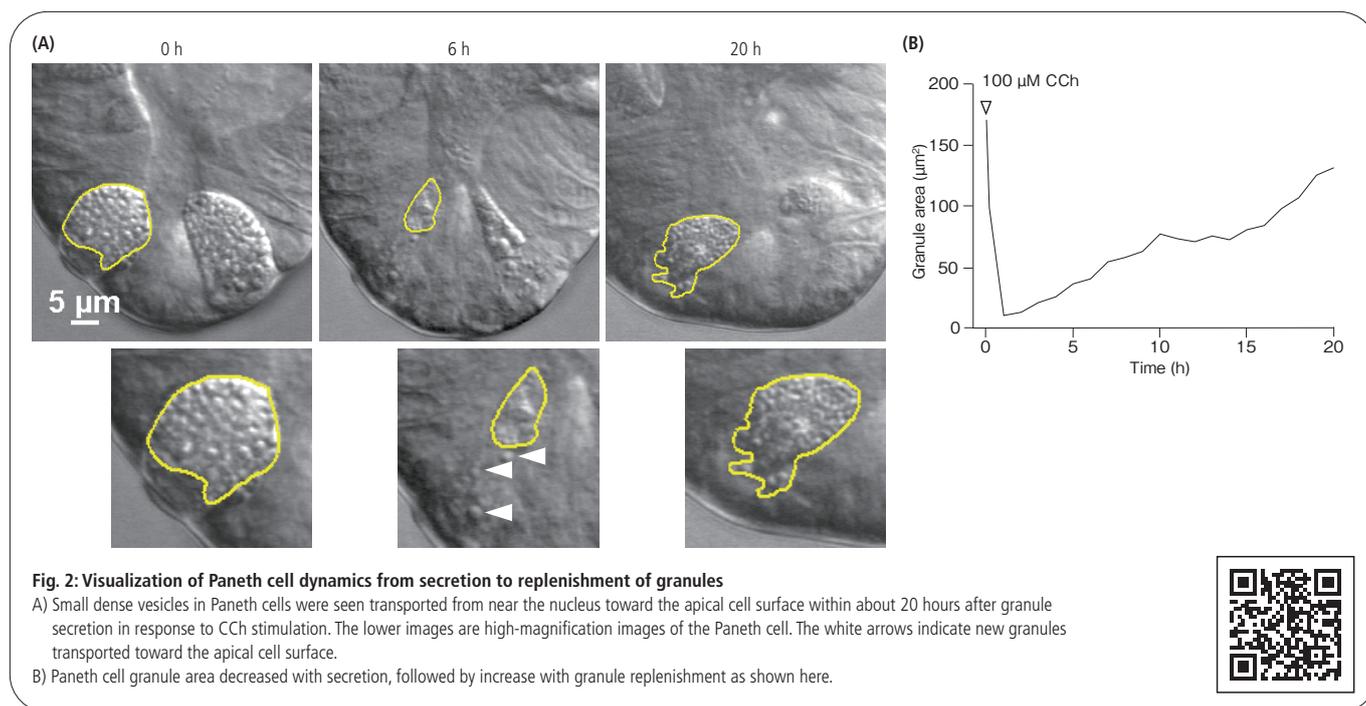
B) To measure Paneth cell granule volume, three-dimensional images of Paneth cell granules stained by Zinpyr-1 were binarized.

C) The volume of Paneth cell granules decreased in response to CCh in a time-dependent manner.



## Long-term tracking of Paneth cell granule replenishment after secretory responses

Paneth cell granule replenishment happening within 20 hours after granule secretion was observed by long-term time-lapse imaging with differential-interference contrast (Fig. 2 (A)). In addition, the process of the secretion and the replenishment of Paneth cell granules in innate enteric immunity was evaluated quantitatively by measuring the granule area (Fig. 2 (B)).



## Summary

In this study, we evaluated Paneth cell dynamics by visualization and quantification for the first time, from granule secretion in response to cholinergic stimulation until replenishment of newly generated granules toward the apical side by vesicular trafficking within 20 hours, and clarified dynamics of Paneth cells in innate enteric immunity. In future, we would like to reveal underlying molecular mechanisms of Paneth cell secretory responses by three-dimensional calcium imaging *ex vivo* and *in vivo*.

## Acknowledgement

Nikon Corporation expresses its sincere thanks to Dr. Yuki Yokoi, Dr. Kiminori Nakamura and Dr. Tokiyoshi Ayabe at Innate Immunity Laboratory, Department of Cell Biological Science, Faculty of Advanced Life Science, Hokkaido University, for providing images and data.

This study was jointly conducted by Hokkaido Univ. and System Development Department, Nikon corporation.

## Reference

Yokoi Y, Nakamura K, Yoneda T, Kikuchi M, Sugimoto R, Shimizu Y, Ayabe T. *Scientific Reports* 9, 2710 (2019).

## Product Information

### A1R HD25 Confocal Microscope

This resonant scanner, which enables high-speed acquisition of up to 720 frames per second, has low phototoxicity toward living cells and is capable of high-speed 3D imaging in combination with a piezo Z stage.

NIS-Elements imaging software provides total support from image acquisition to image analysis.



Piezo Z stage



### CFI Apochromat LWD Lambda S 20XC WI

This lens provides a long working distance as well as a high numerical aperture. It has chromatic aberration corrected over a broad wavelength range from visible to IR. Nano Crystal Coat is applied to increase transmittance. It is capable of capturing bright, sharp images into deep areas in thick living samples such as 3D culture systems and tissue sections.

